

CLAIMS

What is claimed is:

1. An isolated polynucleotide comprising a first nucleotide sequence encoding a polypeptide of at least 494 amino acids that has at least 80 % identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2 and 4 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.

2. The isolated polynucleotide of Claim 1, wherein the first nucleotide sequence consists of a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1 and 3 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2 and 4.

3. The isolated polynucleotide of Claim 1 wherein the nucleotide sequences are DNA.

4. The isolated polynucleotide of Claim 1 wherein the nucleotide sequences are RNA.

5. A chimeric gene comprising the isolated polynucleotide of Claim 1 operably linked to suitable regulatory sequences.

6. A host cell comprising the chimeric gene of Claim 5.

7. A host cell comprising an isolated polynucleotide of Claim 1.

8. The host cell of Claim 7 wherein the host cell is selected from the group consisting of yeast, bacteria, plant, and virus.

9. A virus comprising the isolated polynucleotide of Claim 1.

10. A polypeptide of at least 494 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide selected from the group consisting of SEQ ID NOs:2 and 4.

11. A method of selecting an isolated polynucleotide that affects the level of expression of a flavonoid biosynthetic enzyme polypeptide in a plant cell, the method comprising the steps of:

(a) constructing an isolated polynucleotide comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from an isolated polynucleotide of Claim 1;

(b) introducing the isolated polynucleotide into a plant cell;

(c) measuring the level of a polypeptide in the plant cell containing the polynucleotide; and

(d) comparing the level of polypeptide in the plant cell containing the isolated polynucleotide with the level of polypeptide in a plant cell that does not contain the isolated polynucleotide.

12. The method of Claim 11 wherein the isolated polynucleotide consists of a nucleotide sequence selected from the group consisting of SEQ ID NOs:1 and 3 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2 and 4.

13. A method of selecting an isolated polynucleotide that affects the level of expression of a flavonoid biosynthetic enzyme polypeptide in a plant cell, the method comprising the steps of:

- (a) constructing an isolated polynucleotide of Claim 1;
- (b) introducing the isolated polynucleotide into a plant cell; and
- (c) measuring the level of polypeptide in the plant cell containing the polynucleotide to provide a positive selection means.

14. A method of obtaining a nucleic acid fragment encoding a flavonoid biosynthetic enzyme polypeptide comprising the steps of:

- (a) synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1 and 3 and the complement of such nucleotide sequences; and
- (b) amplifying a nucleic acid sequence using the oligonucleotide primer.

15. A method of obtaining a nucleic acid fragment encoding a flavonoid biosynthetic enzyme polypeptide comprising the steps of:

- (a) probing a cDNA or genomic library with an isolated polynucleotide comprising at least one of 30 contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3 and the complement of such nucleotide sequences;
- (b) identifying a DNA clone that hybridizes with the isolated polynucleotide;
- (c) isolating the identified DNA clone; and
- (d) sequencing the cDNA or genomic fragment that comprises the isolated DNA clone.

16. A composition comprising the isolated polynucleotide of Claim 1.

17. A composition comprising the isolated polypeptide of Claim 10.

18. An isolated polynucleotide comprising the nucleotide sequence having at least one of 30 contiguous nucleotides derived from a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3 and the complement of such sequences.

19. An expression cassette comprising an isolated polynucleotide of Claim 1 operably linked to a promoter.

20. A method for positive selection of a transformed cell comprising:

- (a) transforming a host cell with the chimeric gene of Claim 5 or an expression cassette of Claim 19; and

(b) growing the transformed host cell under conditions which allow expression of the polynucleotide in an amount sufficient to complement a mutant cell with altered isoflavone 2-hydroxylase activity to provide a positive selection means.

21. The method of any one of Claims 11 or 13 wherein the plant cell is a monocot.
- 5 22. The method of any one of Claims 11 or 13 wherein the plant cell is a dicot.
23. An isolated polynucleotide comprising a first nucleotide sequence encoding a polypeptide of at least 141 amino acids that has at least 80 % identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:6 or a second nucleotide sequence comprising the complement of the first nucleotide sequence.
- 10 24. A polypeptide comprising at least 141 amino acids that has at least 80% identity based on the Clustal method of alignment when compared to a polypeptide of SEQ ID NO:6.

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